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1. Your reference AP6

2. Patent application number *(The Patent Office will fill in this part)* **9903403.5** 16 FEB 1999

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Angiogene Pharmaceuticals Ltd
14 Howden Park
Aston Rowant
Watlington OXON OX9 5SX

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation Scotland 7244478001

4. Title of the invention Substituted Stilbene Compounds with Vascular Damaging Activity

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

10 Aston Park
Aston Rowant
Watlington OXON OX9 5SW

Patents ADP number *(if you know it)* 7244478002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
|---|---|---|

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

| Number of earlier application | Date of filing *(day / month / year)* |
|---|---|

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

*a) any applicant named in part 3 is not an inventor, or*
*b) there is an inventor who is not named as an applicant, or*
*c) any named applicant is a corporate body.*
*See note (d))*

Yes

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| | |
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| Continuation sheets of this form | |
| Description | 11 |
| Claim(s) | |
| Abstract | |
| Drawing(s) | |

10. If you are also filing any of the following, state how many against each item.

| | |
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| Priority documents | |
| Translations of priority documents | |
| Statement of inventorship and right to grant of a patent *(Patents Form 7/77)* | |
| Request for preliminary examination and search *(Patents Form 9/77)* | |
| Request for substantive examination *(Patents Form 10/77)* | |
| Any other documents *(please specify)* | |

11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 15/2/99

12. Name and daytime telephone number of person to contact in the United Kingdom Peter Davis 01844 354562

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# SUBSTITUTED STILBENE COMPOUNDS WITH VASCULAR DAMAGING ACTIVITY

This invention relates to vascular damaging agents and particularly to a series of novel stilbene compounds.

Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J Folkman, New England Journal of Medicine 333, 1757-1763, 1995). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy. In all these diseases reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect.

Combretastatin A4 phosphate is an agent known to have vascular damaging activity in animal models of solid tumours (Dark et al, Cancer Research 57, 1829-1834, 1997). However some tumours are resistant to this agent and doses approaching the maximum tolerated dose are necessary to produce significant vascular damage in these tumours.

One characteristic of tumours resistant to combretastatin A4 phosphate is their ability to produce large amounts of nitric oxide. The role of nitric oxide in tumour growth is unclear and there have been reports of both tumour-stimulating and tumour-inhibiting effects (Chinje and Stratford, Essays Biochem. 32, 61-72, 1997).

The present invention concerns novel combretastatin derivatives, methods for their preparation , pharmaceutical compositions containing them and their use as vascular damaging agents for the treatment of diseases involving active angiogenesis. These derivatives are more active than combretastatin A4 or combretastatin A4 phosphate, particularly on tumours that are resistant to the known vascular damaging agents. Though not limiting on the invention it is believed that the ability of the novel compounds to reduce the production of nitric oxide during vascular damage by inhibition of the enzyme which produces it, nitric oxide synthase, is one way in which the compounds achieve increased activity.

Thus in one embodiment of the invention there is provided a compound of formula I

**A-X-B**

**I**

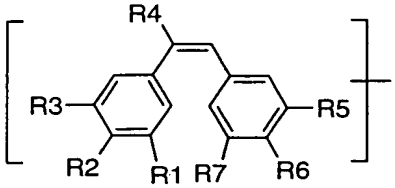
Wherein

A is a substituted *cis*-stilbene moiety
X is a linker group or atom
B is a moiety derived from an inhibitor of nitric oxide synthase

and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

The linker group X can be attached to any available atom of the stilbene moiety A and to any available atom of nitric oxide synthase inhibitor B as appropriate.

The stilbene moiety A can be for example a group of formula II




II

Wherein

R1, R2 and R3 are each independently H, optionally substituted alkoxy, optionally substituted alkyl or halogen
R4 is hydrogen or cyano
R5, R6 and R7 are each independently H, hydroxy, optionally substituted alkoxy, optionally substituted alkyl, halogen, amino, alkylamino, dialkylamino, cyano, nitro, carboxyl, alkanoyl, alkoxycarbonyl, alkoxycarbonyloxy, alkoxycarbonylamino, aminocarbonylamino, alkylaminocarbonylamino, dialkylaminocarbonylamino, alkylcarbonylamino, alkylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl, alkylsulphonylamino, aminosulphonylamino, alkylaminosulphonylamino, dialkylaminosulphonylamino, mercapto, alkylsulphanyl or alkylsulphinyl,

with the proviso that at least two of R1, R2 and R3 must be optionally substituted alkoxy.

Stilbene moiety A can be attached to linker group X by any available valency.

Linker group X can be for example a bond, an optionally substituted methylene chain, or $-(CH_2)_m-Y-(CH_2)_n-$ wherein Y is selected from -O-, -S-, -S(O)-, $-SO_2-$,-NH-, -Nalkyl-, -CO-, -OC(O)-, -NHC(O)-, -N(alkyl)C(O)-, -NHC(O)NH-, -NalkylC(O)NH-, -NalkylC(O)Nalkyl-, $-NHSO_2-$, $-NalkylSO_2-$, $-NHSO_2NH-$, $-NalkylSO_2NH-$, $-NalkylSO_2Nalkyl-$ and -OC(O)O-, m is 0-3 and n is 0-3. Where the group Y is not symmetrical it can be oriented in either direction such that either end can be attached to the group A.

The nitric oxide synthase inhibitor moiety B can be a group derived from any inhibitor of nitric oxide synthase known in the art, for example a group derived from an amino acid inhibitor of nitric oxide synthesis for example a group $-C(O)CH(NH_2)-(CH_2)p-NHC(NH)Z$ wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio, or for example a group $-NHCH(CO_2H)-(CH_2)p-NHC(NH)Z$ where p and Z are as hereinbefore described.

As used herein the term "alkyl", alone or in combinations, means a straight or branched-chain alkyl group containing from one to seven, preferably a maximum of four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl. Examples of alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy and t-butoxy. The term "halogen" means fluorine, chlorine, bromine or iodine.

The term "aryl" as used herein unless otherwise stated includes reference to a $C_{6-10}$ aryl group which may, if desired, carry one or more substituents selected from halogeno, alkyl, haloalkyl, alkoxy, hydroxy, amino, nitro and cyano. The term "aralkoxy" means an alkoxy group substituted with an aryl group.

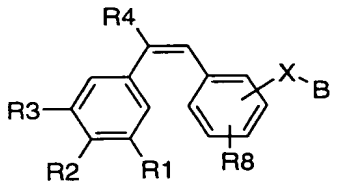
The term heteroaryl is defined herein as a mono- or bi-cyclic aromatic group containing one to four heteroatoms selected in any combination from N, S or O atoms and a maximum of 9 carbon atoms. Examples of heteroaryl groups include pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl , pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl groups.

The term heterocycloalkyl includes heterocycloalkyl groups containing 3-6 carbon atoms and one or two oxygen, sulphur or nitrogen atoms. Particular examples of such groups include azetidinyl, pyrrolidinyl, piperidinyl, homopiperidinyl, piperazinyl, homopiperazinyl, morpholinyl or thiomorpholinyl groups.

The term cycloalkyl means a cycloaliphatic group containing 3-10 carbon atoms such as, for example, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl.

Optionally substituted alkoxy groups , optionally substituted alkyl groups and optionally substituted methylene chains may bear one or more substituents independently selected from halogen, hydroxy, amino, alkylamino, dialkylamino, carboxyl, mercapto, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylcarbonylamino, alkylcarbonyl(alkyl)amino, sulphate and phosphate.

One group of preferred compounds are those of formula III




**III**

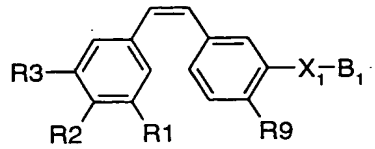
Wherein

R1, R2, R3, R4, X and B are as hereinbefore described
R8 is alkyl, amino, hydroxy, alkoxy or halogen

A further preferred group of compounds are those of formula III wherein R1, R2, R3, R4, are as hereinbefore described, R8 is alkyl, amino, hydroxy, alkoxy or halogen, X

is -O- or -NH- and B is a group -C(O)CH($NH_2$)-($CH_2$)p-NHC(NH)Z wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio or a group -NHCH($CO_2H$)-($CH_2$)p-NHC(NH)Z where p and Z are as hereinbefore described.

A still further preferred subset are compounds of formula IV




**IV**

Wherein
R1, R2 and R3 are as hereinbefore described
R9 is alkyl, alkoxy or halogen
$X_1$ is O or NH
$B_1$ is a group -C(O)CH($NH_2$)-($CH_2$)p-NHC(NH)Z wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio.

Particularly preferred compounds are:

1-(4-methoxy-3-($N^G$-nitroarginylamino)phenyl)-2-(3,4,5-trimethoxyphenyl)-ethene

1-(4-methoxy-3-($N^G$-nitroarginyloxy)phenyl)-2-(3,4,5-trimethoxyphenyl)-ethene

1-(4-methoxy-3-($N^G$-methylarginylamino)phenyl)-2-(3,4,5-trimethoxyphenyl)-ethene

1-(4-methoxy-3-($N^G$-methylarginyloxy)phenyl)-2-(3,4,5-trimethoxyphenyl)-ethene

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by "hereinbefore defined" or "defined hereinbefore", or "hereinafter defined" or "defined hereinafter", the said group encompasses the first occurring and broadest definition as well as each and all of the preferred definitions for that group.

Where one or more functional groups in compounds of formula I are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates, salts derived from inorganic bases including alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and salts derived from organic amines such as morpholine, piperidine or dimethylamine salts.

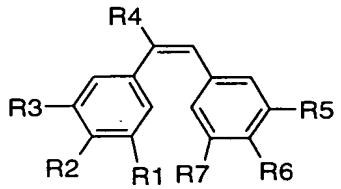
Compounds of formula I or a salt thereof may exhibit tautomerism and the formulae drawings within this specification represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form that has vascular damaging activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

Those skilled in the art will recognise that compounds of formula I may exist as stereoisomers and accordingly the present invention includes all such isomers and mixtures thereof.

Compounds of the invention can be prepared by any process known to a person skilled in the art. Compounds of formulae I, III and IV can be prepared by a number of processes as generally described hereinbelow and more specifically in the Examples hereinafter. In the general preparations described below it may be necessary to employ protecting groups which are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art. In the following process description, the symbols R1, R2, R3, R4, R5, R6, R7, X and B when used in the formulae depicted are to be understood to represent those groups described above in relation to formula I unless otherwise indicated

Thus according to a further aspect of the invention compounds of the invention may be prepared by attachment of a nitric oxide synthase inhibitor to a stilbene of formula V using alkylation, acylation, sulphonylation or coupling reactions. Alternatively stilbenes of formula V may be coupled to a difunctional compound (which provides the linker group -X-) and further coupled to the nitric oxide inhibitor via the remaining functionality on the linker group as appropriate. Stilbenes of formula V are either known or can be prepared using methods analagous to those used in the preparation of the known stilbenes which will be apparent to those skilled in the art.

In one general example compounds of formulae I can be prepared from a stilbene of formula V containing a free OH or NH by acylation with a nitric oxide synthase inhibitor containing a carboxylic acid for example using a coupling agent such as a carbodiimide, for example dicyclohexylcarbodiimide, or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and, optionally, a base such as an organic base for example triethylamine and, optionally, a catalyst such as 4-dimethyaminopyridine in a solvent such as an aprotic solvent for example dimethylformamide or in a chlorinated solvent for example chloroform or dichloromethane at a temperature in the range from about -30°C to about 60°C, conveniently at or near room temperature.



V

In another general example a compound of formula V containing a free OH or NH group can be treated with phosgene or a phosgene equivalent such as 1,1′-carbonyldiimidazole or triphosgene in the presence of a base such as an organic base for example triethylamine, pyridine or N-methylmorpholine in a solvent such as an ether solvent for example tetrahydrofuran or in a chlorinated solvent for example dichloromethane at a temperature in the range from about -20°C to the reflux

temperature of the solvent, followed by treatment with a nitric oxide inhibitor containing a free OH or NH group to give a compound of formula I containing a carbonate, carbamate or urea group.

In another general example a compound of formula V containing a free NH group can be treated with a dicarboxylic acid monoester such as monomethyl succinate in the presence of a coupling agent such as a carbodiimide, for example dicyclohexylcarbodiimide, or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and, optionally, a base such as an organic base for example triethylamine in a solvent such as an aprotic solvent for example dimethylformamide or in a chlorinated solvent for example chloroform or dichloromethane at a temperature in the range from about -30°C to about 60°C, conveniently at or near room temperature. The resulting ester can be hydrolysed by treatment with aqueous acid or aqeuous base under standard conditions and the carboxylic acid so obtained treated with a nitric oxide inhibitor containing a free OH or NH group, using a coupling agent as described hereinbefore, to give compounds of the invention.

In another general example a compound of formula V containing a carboxylic acid group can be converted into a compound of formula I containing an amide or ester by treatment with a nitric oxide synthase inhibitor, containing an amino group or a hydroxyl group respectively, using a coupling agent as described hereinbefore.

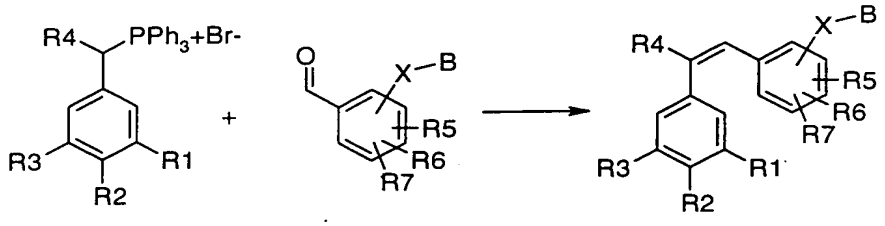
In another general example a compound of formula V containing a monohaloalkyl group can be reacted with a nitric oxide synthase inhibitor containing a free OH, NH, or SH group in the presence of a base such as sodium carbonate or a metal hydride such as sodium hydride in a solvent such as dimethylformamide at a temperature of about 0°C to a temperature of about 100°C to give compounds of the invention.

In another general example a compound of formula V containing a carboxylic acid group can be treated with a monoprotected diamino, dihydroxy or aminohydroxy compound such as a monoprotected diaminoalkane, a monoprotected dihydroxyalkane or mono-protected aminohydroxyalkane, using a coupling agent as described hereinbefore and the resulting amide or ester deprotected and reacted with a nitric oxide synthase inhibitor containing a carboxylic acid using a coupling agent as described hereinbefore.

In another general example a compound of formula V containing a free OH or NH group can be sulphonylated with a protected amino sulphonylchloride such as a protected aminoalkylsulphonylchloride or a protected hydroxy sulphonyl chloride such as a protected hydroxyalkylsulphonyl chloride, in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature and the resulting sulphonamide or sulphonate deprotected and reacted with a nitric oxide synthase inhibitor containing a carboxylic acid using a coupling agent as described hereinbefore.

In another general example a compound of formula V containing a free OH, SH or NH group can be alkylated with a difunctional alkylating agent such as a dihaloalkane in the presence of a base such as sodium carbonate or a metal hydride such as sodium hydride in a solvent such as dimethylformamide at a temperature of about 0°C to a temperature of about 100°C, and the resulting haloalkane further reacted under similar conditions with a nitric oxide synthase inhibitor containing a free OH, SH or NH group.

Compounds of formula VII can also be prepared by Wittig olefin synthesis involving reaction of a phosphonium salt of formula VI with a strong base, for example an alkyllithium such as n-butyllithium or t-butyllithium or a metal hydride such as sodium hydride in a solvent such as an ether solvent for example diethyl ether or tetrahydrofuran or in a solvent such as a hydrocarbon solvent for example toluene at a temperature of between about -100°C to about 30°C followed by treatment with an aldehyde of formula VII.




VI VII VIII

Compounds of formula I can also be prepared from other compounds of formula I by chemical modification. Examples of such chemical modifications that may be applied are standard alkylation, arylation, heteroarylation, acylation, thioacylation, sulphonylation, sulphation, phosphorylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formula I may be modified by, for example, oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formula I.

Thus for example a compound of formula I containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In another general example of an interconversion process an amino group in a compound of formula I may be sulphonylated by treatment with, for example, an alkyl or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In a further general example a compound of formula I containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with for example di-tert-butyl diisopropylphosphoramidite in the presence of a suitable catalyst for example tetrazole in a solvent such as an ether solvent for example tetrahydrofuran at a temperature in the range -40 to 40°C, conveniently at or near room temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid at a temperature in the range -78°C to 40°C preferably -40 to -10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30 to 40°C conveniently at or near 0°C to give the compound of formula I containing a dihydrogenphosphate ester.

In a further general example a compound of formula I containing an amide can be hydrolysed by treatment with for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

In another general example an O-alkyl group may be cleaved to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example compounds of formula I may be alkylated by reaction with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of around -10 to 80°C.

Preparation of a compound of formula I as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

Acid addition salts of the compounds of formula I are prepared in a conventional manner by treating a solution or suspension of the free base I with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formula I derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension of the free acid I with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallisation techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy tumour vasculature and vasculature that has been newly formed while leaving unaffected normal, mature

vasculature. The ability of the compounds to act in this way may be determined by the tests described in the Examples hereinafter.

The compounds according to the invention are thus of particular use in the prophylaxis and treatment of cancers involving solid tumours and in the prophylaxis and treatment of diseases where inappropriate angiogenesis occurs such as diabetic retinopathy, psoriasis, rheumatoid arthritis, atherosclerosis and macular degeneration.

The compounds of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide; antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example aspariginase; topoisomerase inhibitors for example etoposide, topotecan and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

For the prophylaxis and treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions selected with regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutical compositions may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician but in general daily dosages may be in the range 0.001 to 100mg/kg preferably 0.01 to 50mg/kg.

BIOLOGICAL ACTIVITY

The following tests were used to demonstrate the activity and selectivity of compounds according to the invention.

<u>Activity against tumour vasculature measured by radioactive tracer.</u>

The following experiment demonstrates the ability of the compounds to damage selectively tumour vasculature.
Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately $10^6$ cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10 g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour kidney, liver, skin muscle, gut and brain by the $^{86}$RbCl extraction technique (Sapirstein, Amer J Physiol, **193**, 161-168, 1958). Tissue radioactivity measured 1 minute after an intravenous injection of $^{86}$RbCI was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit J Radiol., **55**, 905-913, 1982). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

Activity against tumour vasculature measured by fluorescent dye.
The following experiment further demonstrates the ability of the compounds to damage tumour vasculature.
Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith *et al* (Brit J Cancer **57**, 247-253, 1988). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25 mg/ml and injected intravenously at 10 mg/kg 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10 µm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (J Natl Cancer Inst, **4**, 47-53, 1943). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.
A similar procedure was used to estimate perfused vascular volume in Sarcoma S bearing mice (Parkins et al. Cancer Res. 55, 6026-9, 1995) with the exception that an alternative fluorescent dye was used. In this case the dye 3,3'-diheptyloxacarbocyanine (Molecular Probes Inc. Eugene, OR, USA) was dissolved in 3:1 dimethylsulphoxide:saline to a final concentration of 0.6mg/ml and injected intravenously at a dose of 1mg/kg body weight. Sections were visualised using 490nm excitation with a 520nm filter and scored as above.

The following non-limiting Example illustrates the invention:

EXAMPLE 1
1-(4-Methoxy-3-$N^G$-nitroarginyloxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene

Trifluoroacetic acid (0.2ml) was added to a solution of 1-(3-(N-α-t-butoxycarbonyl-N-ω-nitroarginyloxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene in dichloromethane (3ml) at 0°C and the mixture allowed to come to room temperature and stir 16h. The mixture was concentrated under reduced pressure, ethanol (5ml) was added, the mixture was reconcentrated under reduced pressure and the procedure

repeated three times. Trituration with diethyl ether afforded the title compound (69mg) as an off-white powder m.p. 157-159°C.

The 1-(3-(N-α-t-butoxycarbonyl-N-ω-nitroarginyloxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene used in the above procedure was prepared as follows:
A solution of 1-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (65mg, 0.21mmol), Nα-t-BOC-ω-nitro-L-arginine (134mg, 0.42mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (110mg, 0.54mmol) and 4-dimethylaminopyridine (5mg) in dichloromethane (2.1ml) was stirred at room temperature for 72h. The reaction mixture was partitioned between dichloromethane and water and the aqueous phase extracted with two portions of dichloromethane. The combined organic extracts were washed successively with two portions of water and one of brine, dried (MgSO4) and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with 33% ethyl acetate/hexane followed by 100% ethyl acetate to give 1-(3-(N-α-t-butoxycarbonyl-N-ω-nitroarginyloxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (82mg) as a white oil.

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